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Effects of fragmentation and seawater submergence on photochemical efficiency and growth in the clonal invader *Carpobrotus edulis*

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Highlights

- Clonal propagation has been suggested as important traits for plant invasions
- Carbohydrates stored in the stolon can be mobilized to buffer stress conditions
- We simulated fragmentation and sea-water submergence in a clonal invader
- Stolons, as a source of carbohydrates, allows the expansion of clonal invaders

Abstract

Clonal plants are frequently affected by process of disturbance as fragmentation. The capacity of these fragments to survive and grow after disturbance has important implications for the expansion of clonal plants, and could have special consequences for the colonization of new environments by invasive clonal species. Stolon internodes of clonal plants represent important reserve organs. These storage structures can play a crucial role in the survival and re-growth of clonal plants after an event of disturbance. In this study we simulated physical disturbance by fragmentation of clones of the stoloniferous invader *Carpobrotus edulis* into ramets with short and long stolon lengths, and a subsequent event of seawater submergence and de-submergence. Ramets with long stolons showed a significantly higher total biomass than ramets with short stolons, supporting the idea that stolon length is related with the amount of reserves stored and with the benefit reported in terms of growth. Our results showed that the benefit of having long stolons was also important for clonal fragments that suffered a process of seawater submergence. Our study suggests that the use of stolon as a source of resources can represent a suitable mechanism for colonization of coastal sand dunes by the aggressive invader *C. edulis*.

Key-words: chlorophyll fluorescence, clonal growth, fragmentation, disturbance, plant invasions, seawater submergence, spectral reflectance, stolon length, storage organ.

1. Introduction

Clonal propagation allows the production of a number of ramets that can remain physically connected through stolons or rhizomes, forming large structures that may occupy considerable areas (Cain, 1997; Hutchings et al., 2004; Klimes et al., 1997; Oborny and Price and Marshall, 1999). These large clonal structures may frequently be affected by processes of disturbance that tear them up into fragments of different size (Barrat-Segretain and Bornette, 2000; Latzel and Klimesŏvá, 2009; Stuefer and Huber, 1999). The capacity of these fragments to survive and re-grow after disturbance has important implications for the expansion of clonal plants, and could play a crucial role in the colonization of new environments by invasive clonal species (Dong et al., 2010, 2012; Konlechner et al., 2016; Lin et al., 2012; Song et al., 2013a;). In particular, in rocky coasts, clonal structures as rhizomes or stolons can be very often fragmented by intense wave action (Maun, 1994), and long-distance transported by sea waves, representing an important dispersion strategy after tempests (Aptekar and Marcel, 2000; de la Peña et al., 2011; Harris and Davy, 1986a, 1986b; Huiskes, 1979; Konlechner and Hilton, 2009). Specifically, in coastal sand dunes species, it has been showed that both seeds and clonal structures play an important role in the colonization of new environments (Harris and Davy, 1986a, 1986b). Even more, plant establishment on coastal sand dune can be more successful from clonal

fragments than from seeds, because of the large amount of stored reserves in clonal organs (Maun, 2009).

Seawater submergence can be a common situation for plants inhabiting rocky coast and foredune habitats. Water submergence usually produces a photosynthetic collapse, due to critical reduction of light and gas exchange, with the resulting energy crisis for the plant. Generally, after the energy crisis the plant will suffer a depletion of carbohydrates that cannot be fully restocked by the acceleration of glycolysis or the induction of fermentative metabolism (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009; Sairam et al., 2008;). Damages caused by submergence in the plant can continue after de-submergence due to post-anoxic injury (Sarkar et al., 2006). In addition, seawater can induce salt injure on chloroplasts, affecting electron transport, and consequently reducing photosynthetic efficiency (Gao et al., 2015; Larcher, 1995). Saltwater increase the concentration of Na^+ and Cl^- in plant tissues, altering ionic ratios in plants and producing ion toxicity (Barrett-Lennard and Shabala, 2013; Grattan and Grieve, 1998; Rhoades et al., 1999). As a result, saltwater can have a negative impact on growth, compromising plant development and survival (Barrett-Lennard and Shabala, 2013; Im et al., 2014)

Stolon connections allow clonal plants to be physiologically integrated (i.e. to shareresources between connected modules). During the last decades, many studies have been oriented to explore the benefits of this integration for the colonization of a wide variety of environments by clonal plants (e.g. Alpert and Mooney, 1986; Alpert, 1999; Hartnett and Bazzaz, 1983; Roiloa et al., 2014c; Roiloa and Retuerto, 2006; Slade and Hutchings, 1987). However, the potential benefits for clonal plants of the reserves storage in the stolons have been less explored (but see; Dong et al., 2012, 2011, 2010; Isogimi et al., 2014; Lin et al., 2012; Stuefer and Huber, 1999; You et al.,

2014). By storing resources, stolons can play a decisive role in the survival and re-growth of clonal plants after disturbance events. Resources stored in the stolon can be mobilized helping to buffer stress conditions (Goulas et al., 2001; Stuefer and Huber, 1999; Suzuki and Stuefer, 1999), and therefore can represent an additional benefit from clonal attributes.

Carpobrotus edulis L. is a stoloniferous invader that inhabits coastal habitats wheretheir clonal clumps may experience natural disturbances, as tempests, with fragmentation of the clonal system in pieces of different size, These may be wash down by the sea and later returned to land. In this study, we simulate physical disturbance in *C.edulis* by fragmenting their clones into ramets with short and long stolon lengths, and a subsequent event of seawater submergence and de-submergence. The objective of this study is to determine the role of stolons as storage organs in maintaining photochemical activity and growth of *C. edulis* clones after fragmentation and seawater submergence. Recent studies have been conducted to determine the importance of clonal integration (i.e. resource sharing between connected modules of the clone via stolons) in the expansion of *C. edulis* (Roiloa et al., 2014a, 2014b, 2013, 2010). However, the role of clonal structures as storage organs and their potential contribution to the expansion of this invader has not yet been investigated.

Specifically, we aim to respond to the following questions: (1) does stolon length affect photochemical activity and growth of *C. edulis* after physical fragmentation? Because stolons can act as reserve organs (Goulas et al., 2001; Stuefer and Huber, 1999; Suzuki and Stuefer, 1999), and the stolon length may be positively correlated with the amount of reserves stored (Dong et al., 2010), we expect that increased stolon length will positively affect the photochemical activity and growth of the fragmented ramets. (2) Are growth and photochemistry of longer ramets less

affected by a stressful event of seawater submergence? Because reserve storage could be critical to mitigate the carbohydrate crisis due to submergence (Striker, 2012), we predict that the benefits of increased stolon length, in terms of growth and photochemical activity, will be greater for ramets subjected to seawater submergence.

2. Material and methods

2.1. Study species

Carpobrotus edulis (L.) N.E. Br. is a mat-forming succulent plant native to the Cape (South Africa), and currently an aggressive invader in all the Mediterranean climate areas around the world, where it colonizes rocky coast and coastal sand dunes (D'Antonio, 1993; D'Antonio and Mahall, 1991; Traveset et al., 2008; Vilà et al., 2008). *C. edulis* propagates clonally by the production of stolons, showing a radial growth with a structure of nodes and internodes that allows an effective colonization of the surrounding area (Wisura and Glen, 1993).

2.2. Experimental design

Forty similar size un-rooted ramets of *C. edulis* were collected in a dune system in Quiaios (Portugal) (40°13'N, 8°53'W). Each ramet was obtained by excising the fourth unit from the apex of a maternal clump. Normally, the fourth unit from the apex represents in *C. edulis* a well-developed ramet. This protocol allowed to standardize the age, size and developmental stage of the plant material used in the experiment. Ramets were collected from a total of 10 maternal clumps (4 ramets from each) separated from each other by at least 50 m. Because plant material was collected over a relatively large area, we assume that each clump represents a different genotype. The experimental design included two crossed factors: 'stolon

length' (short, long) and 'seawater submergence' (submerged, non submerged) (see Fig. 1). To test the effect of stolon length as a storage organ we prepared ramets with short (2.11 ± 0.09 cm, mean ± SE) and long (9.06 ± 0.18 cm, mean ± SE) stolons by cutting the appropriated length with scissors. We did not observe any negative effect of cutting the stolon (as sudden death or diseases). Long and short stolons significantly differed in length ($F_{1,38}$ = 1095.752, P < 0.001), but not in initial fresh biomass of the ramets ($F_{1,38}$ = 3.041, P = 0.089). To test the effect of seawater submergence on plant performance, half of the ramets in each 'stolon length' treatment were seawater submerged during 48h in a unique cycle, and the other half were not. Submerged ramets (leaves + stolon) where immersed in a 20cm-depth tray with seawater, whereas non-submerged plants remained in a tray without water. We used seawater collected from the Atlantic Ocean, in the proximities where *C. edulis* inhabit (ca. 34 g salts/L water; Na 11 g/L water). After this, all the ramets were planted individually in 5L plastic pots filled with sand from dunes where *C. edulis* inhabits. These treatments imitate natural conditions, where clones of *C. edulis* inhabiting rocky coasts are fragmented into pieces of different size by the action of storm waves, transported along the shore and stranded later on the beaches and foredunes. Although the period of seawater submergence could be quite variable, submergence during 48h is a realistic scenario for *C. edulis*, where plant fragments are abandoned again in the beach after a relatively short period of time. Ramets from each of the original maternal clumps were equally represented in each combination of treatments. Each treatment was replicated 10 times. The experiment was carried out in a greenhouse at the University of Santiago de Compostela (Spain) during 3 months, from March 27 until harvest on June 29. All the ramets were randomly arranged in the

greenhouse. Plants grew under a natural day/night light cycle and were watered regularly to prevent water stress.

2.3. Growth and physiological measurements

Growth: At the end of the experiment, ramets were harvested individually, divided into shoots (leaves and stolons) and roots, oven-dried at 60 °C to constant weight and weighed to the nearest 0.0001 g (Mettler AJ100, Greifensee, Switzerland). Total mass was calculated for each ramet as the sum of shoot and root dry mass. In addition, the proportional biomass allocated to roots was also determined as the ratio root/total mass (root mass ratio, RMR).

Chlorophyll fluorescence: Chlorophyll fluorescence parameters were measured every 30 days from the start of the experiment in all the ramets by the saturation pulse method (Schreiber *et al.* 1998), using a portable fluorometer (MINI-PAM photosynthesis yield analyser; Walz GmbH, Effeltrich, Germany). The maximum (Fv/Fm) and actual (ΦPSII) quantum yield of photosystem II (PSII) were determined as follows: Fv/Fm was calculated as $(Fm - F_0) / Fm$ (Bolhàr-Nordenkampf et al., 1989), where F_0 and Fm are, respectively, the minimal and maximal fluorescence yield of a dark-adapted sample, when all PSII reaction centres are fully open (i.e. all primary acceptors oxidized). The Fv/Fm ratio provides information on the efficiency of excitation energy capture by open PSII reaction centres (Butler and Kitajima, 1975) and is correlated with the amount of carbon gained per unit of light absorbed (Bolhàr-Nordenkampf and Öquist, 1993). On the other hand, ΦPSII was calculated as $(F'_m - F_t) / F'_m$ (Genty et al., 1989), where F'_m is the maximal fluorescence yield reached in a pulse of saturating light with an illuminated sample, and F_t is the fluorescence yield of the leaf at a given photosynthetic photon flux density. This parameter was measured

under the natural ambient light of 285.5±13.4 μmol m^{-2} s^{-1} (mean ± SE, n =120). ΦPSII is a measure of the fraction of the light absorbed by chlorophylls associated with PSII that is used in photochemistry (Maxwell and Johnson, 2000) and can be used to predict CO_2 assimilation rates (Andrews et al., 1995; Edwards and Baker, 1993).

Leaf spectral reflectance: Immediately after the chlorophyll fluorescence measurements, spectral reflectance parameters were determined in all the ramets, using a portable spectrometer (UniSpec Spectral Analysis System; PP Systems, Haverhill, MA, USA). Specifically, we determined the chlorophyll content index (CHL), and the photochemical reflectance index (PRI). CHL was calculated as R750 / R700, where R750 and R700 are reflectances at 750 and 700 nm, respectively. The CHL is significantly correlated with the chlorophyll content of leaves (Lichtenthaler et al., 1996; Wood et al., 1993). PRI was calculated as (R531 - R570) / (R531 + R570), where R531 and R570 are reflectances at 531 and 570 nm, respectively. This index is correlated with net CO_2 uptake and photosynthetic radiation-use efficiency (mol CO_2 /mol photons) (Filella et al., 1996; Gamon et al., 1997 Peñuelas et al., 1995).

2.4. Data analyses

Total mass and RMR were compared by two-way ANOVA, with ‘stolon length’ and ‘seawater submergence’ as between-subject effects. Differences in chlorophyll fluorescence (Fv/Fm and ΦPSII) and spectral reflectance (CHL and PRI) parameters were analysed by repeated measures two-way analysis of variance (ANOVAR), with ‘stolon length’, and ‘seawater submergence’ as between-subject effects and ‘time’ as the within-subject effect. Total mass was log10-transformed to meet the requirements

of parametric statistical tests. Significance levels were set at $P<0.05$. Statistical tests were performed with SPSS 15.0 (SPSS, Chicago, IL, USA).

3. Results

In our study, all the fragments developed roots and survived after the disturbances, denoting the capacity of *C. edulis* to colonize a new environment even after these stressful treatments. Total dry mass of ramets was significantly affected by stolon length and seawater submergence treatments. Short stolon ramets showed a significant lower total mass than long stolon ramets (Table 1, Fig. 2a). Likewise, seawater submergence reduced significantly the total mass of the ramets, and this effect was not dependent on stolon length (Table 1, Fig. 2a). However, the proportional biomass allocated to roots (RMR) was not affected by any of the experimental treatments (Table 1, Fig. 2b).

Seawater submergence significantly reduced the maximum quantum yield of PSII (Fv/Fm) and the chlorophyll content index (CHL) (Table 2, Fig. 3a,c). For the CHL index, the effect of seawater submergence depended on time, being the differences between non-submerged and submerged ramets greater at the middle of the experiment (Fig. 3c). The actual quantum yield of PSII (ΦPSII) and the photochemical reflectance index (PRI) were not significantly affected by 'stolon length', 'seawater submergence' or their interaction (Table 2).

4. Discussion

Our results supported the hypothesis that increased stolon length positively affects the growth of *C. edulis* (hypothesis 1), but did not sustain that this benefit was proportionally greater for seawater submerged ramets (hypothesis 2). Plants growing

in coastal habitats, especially those occupying rocky coasts, are frequently exposed to unpredictable disturbances as tempests (Bauer et al., 2009; Maun, 2009). In the case of clonal plants, the systems of connected ramets are frequently fragmented in pieces of different size, swept away to the sea by the action of waves, and later dropped again to the dune (Aptekar and Marcel, 2000; Harris and Davy, 1986b; Konlechner and Hilton, 2009). In this context, the capacity of the fragments to survive and re-grow after disturbances will be key for the colonization of new environments and for population maintenance. The storage of resources in reserve structures, as stolons, may contribute to increase survival, and therefore it has been considered an advantage for plants inhabiting recurrently disturbed habitats (Goulas et al., 2001; Stuefer and Huber, 1999; Suzuki and Stuefer, 1999;).

In our study, seawater submergence significantly reduced the amount of light-harvesting antenna pigments, as expressed by CHL values, photochemical activity of PSII, as estimated by Fv/Fm, and growth. Similar results have been obtained by Mangora et al. (2014), who found a reduction of Fv/Fm due to submergence in three mangrove species. We demonstrate that chlorophyll fluorescence (Fv/Fm) and spectral reflectance parameters (CHL) can be used as functional indicators of the effect of seawater submergence in processes related to photochemical efficiency. Previous studied have also showed that chlorophyll fluorescence parameters are successful at detecting saltwater flooding stress (Naumann et al., 2008). In addition, our results showed that the negative effect of seawater submergence at physiological level (evidenced by the reduction of the photochemical efficiency) was transferred to a reduction in growth, linking physiological and morphological responses. The negative impact of submergence for plants has been previously reported. Submergence reduces light and diffusion of CO_2 (required in chloroplasts for

photosynthesis) and O_2 (required in mitochondria for respiration), conducting to a reduction of the photosynthetic activity and energy crisis that produce cellular damage (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009; Gibbs and Greenway, 2003). In addition, salinity may alter ionic ratios in plants (Grattan and Grieve, 1998), produce specific ion toxicity (Rhoades et al., 1999), and change the allocation of macro and micronutrients within plant tissues (Hu et al., 2007). All these adverse effects may damage photosynthesis by stomatal closure and by salt-induced injury on chloroplasts, especially on electron transport (Gao et al., 2015; Larcher, 1995;). Abou Jaoude et al. (2013) have found that saltwater flooding damages plants more severely than freshwater flooding, and Naumann et al. (2008) reported that net photosynthesis decreased significantly more after three-days submergence in saltwater compare to freshwater submergence. Interestingly, our results showed a negative effect of seawater in plants that were shallowly submerged for a relatively short period of time, and thus expand previous effects found after a long period of submergence in profundity (Mangora et al., 2014). However, because our design does not include a freshwater submergence treatment, we cannot identify the source (submergence or salinity) of the negative effect encountered in plants under seawater submergence conditions.

Our results showed a significant increase in growth (total biomass) for those ramets fragmented in larger pieces (long stolon treatment), as we predicted in our first hypothesis. Stolons act as important reservoirs for carbohydrate storage, and consequently might be crucial for recovering after a process of fragmentation. Reserves stored in the stolons can be remobilized favoring plant re-growth and helping to buffer the negative impact of disturbance (Goulas et al., 2001; Stuefer and Huber, 1999; Suzuki and Stuefer, 1999). It seems logical that the increase in stolon

length will be positively related with the increase in the amount of resources stored, and consequently with benefits obtained by the plant (Dong et al., 2010). In our study, ramets with long stolons produced a significantly higher total biomass than ramets with short stolons, supporting the idea that stolon length is related with the amount of reserves stored and with the benefit reported in terms of growth. Previous studies also suggested that the variation in the sprouting ability of the clonal invader *Ammophila arenaria* in New Zeland was caused by variations in the growth reserves stored within the rhizomes (Konlechner et al., 2016). In a manipulative experiment with 39 stoloniferous native and invasive plant species Song et al. (2013a) examined the regeneration capacity of single-node fragments with or without attached stolon internodes. Similarly to our results, the presence of stolon internodes increased regeneration rate and subsequent growth. However, although results from Song et al. (2013a) suggest that the capacity for resource storage in stolon internodes may play an important role for clonal plants expansion, it did not seem to differ between native and invasive species. More studies comparing the importance of clonal organs, as storage organs, between exotic invaders and exotic non-invaders, or between species with different degree of invasiveness, would be necessary to better elucidate the contribution of these clonal traits to plant invasions.

Our results also showed that the biomass allocation (estimated by the root mass ratio, RMR) was not significantly affected by the stolon size. In spite of having less reserves, ramets with short stolons did not increase significantly the proportional biomass allocated to roots, as we could expect from the optimal partitioning theory (i.e. plants will increase the proportional biomass allocation to the structures responsible of acquiring the most limiting resource) (Bloom et al., 1985; Thornley, 1972). Instead, root: total biomass maintained invariant scaling relations with stolon

length. Similar results in other species have been interpreted as evidence that evolutionary pressures, more specifically, biomechanical and hydrodynamic constraints, could be acting to maintain allometric ratios at specific values (Sanchez-Vilas and Retuerto, 2007).

The benefit derived from the role of stolons as reserve organs is especially important for clonal plants inhabiting coastal habitats, where the process of disturbance frequently include fragmentation and seawater submergence (Aptekar and Marcel, 2000; de la Peña et al., 2011; Harris and Davy, 1986b; Konlechner and Hilton, 2009). One of the harmful consequences of seawater submergence is the carbohydrate crisis. Photosynthesis activity is collapsed due to submergence and salt stress, and the plant mobilizes the carbohydrates stored to sustain energy production. These carbohydrates are not restocked and their depletion conducts to cell death (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009). Under this situation of stress, the presence of a high accumulation of carbohydrates may be critical to allow the recovery after seawater submergence (Striker, 2012). In this sense, ramets of *C. edulis* with long stolon may buffer the process of fragmentation and seawater submergence, increasing the probability for re-colonization. However, to confirm the mechanism behind this finding, mobilization of stored resources must be traced, allowing a more reliable interpretation of our results. Future studies should trace and quantify the mobilization of internal resources to elucidate the real ecological function of clonal storage structures in plant invasions.

Success in long-distance dispersion (movement of individuals from their birth location to another place that might be colonized, *sensu* Bullock et al., 2002) can imply important consequences for plant invasions (Trakhtenbrot et al., 2005). In coastal habitats, clonal structures can be fragmented and long-distance transported,

representing an important mechanism for dispersion and colonization of new coastal environments (Harris and Davy 1986a, 1986b). In this sense, the use of stolons as storage organs could be playing an important role in the expansion of clonal invaders (Dong et al., 2012, 2011, 2010; Konlechner et al., 2016; Lin et al., 2012). Attributes associate to clonal propagation have been recently suggested as important traits for plant invasions (Liu et al., 2006; Song et al., 2013b). Most of the studies have been focused on the benefits of physiological integration (Song et al., 2013b; Wang et al,. 2008; Yu et al., 2009) for clonal invaders, and recent studies explicitly conducted with *C. edulis* have reported benefits of clonal integration at physiological and morphological level (Roiloa et al., 2016, 2014a, 2014b, 2013, 2010). However, this is the first research exploring the benefit of clonal structures as storage organs for the invader *C. edulis*. Our study suggests that the use of stolons as a source of resources can represent a suitable mechanism for colonization of new coastal dunes habitats by the aggressive invader *C. edulis*. In this regard, our results indicate that *C. edulis* inhabiting rocky coast could be considered as a source of propagules, with potential to be transported along shore by waves and colonize new coastal habitats. This information is particularly interesting for managing plant invasions.

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LEGEND FIGURES

FIG.1 Schematic representation of the experimental treatments with stolon length (short, long) and sea water submergence (submerged, non submerged) as main factors See text for experimental design details.

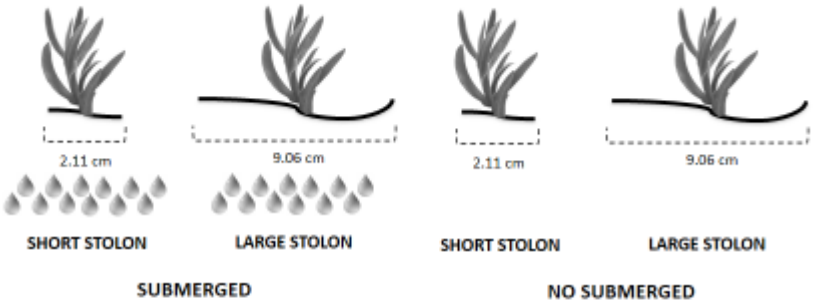

FIG. 2 Total mass in g (mean + SE) (a) and proportional biomass allocated to roots (determined as the root mass ratio, RMR) (mean + SE) (b) of short and long stolon ramets in sea water submerged and non submerged treatments. See Table 1 for ANOVAs results.

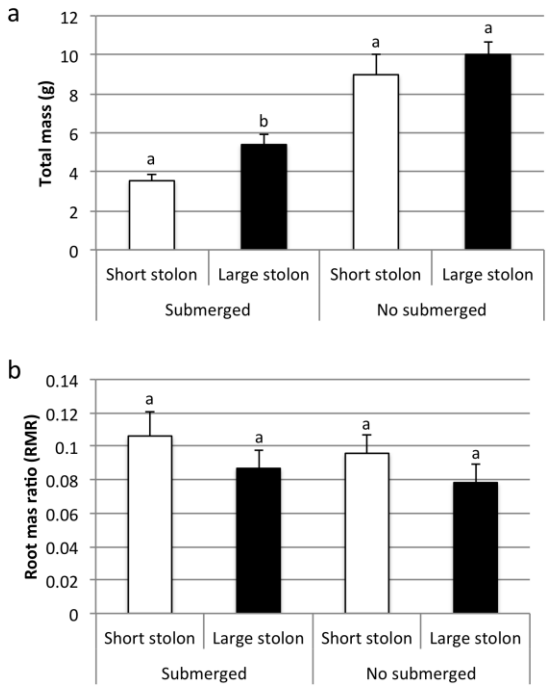

FIG. 3 Time-course of mean values (±SE) of chlorophyll fluorescence (Fv/Fm and ΦPSII, a and b panels respectively) and spectral reflectance (CHL and PRI, c and d panels respectively) parameters for short (dashed lines) and long (solid lines) stolon, and seawater submerged (closed symbols) or non submerged treatments (open symbols). See Table 2 for ANOVAs.

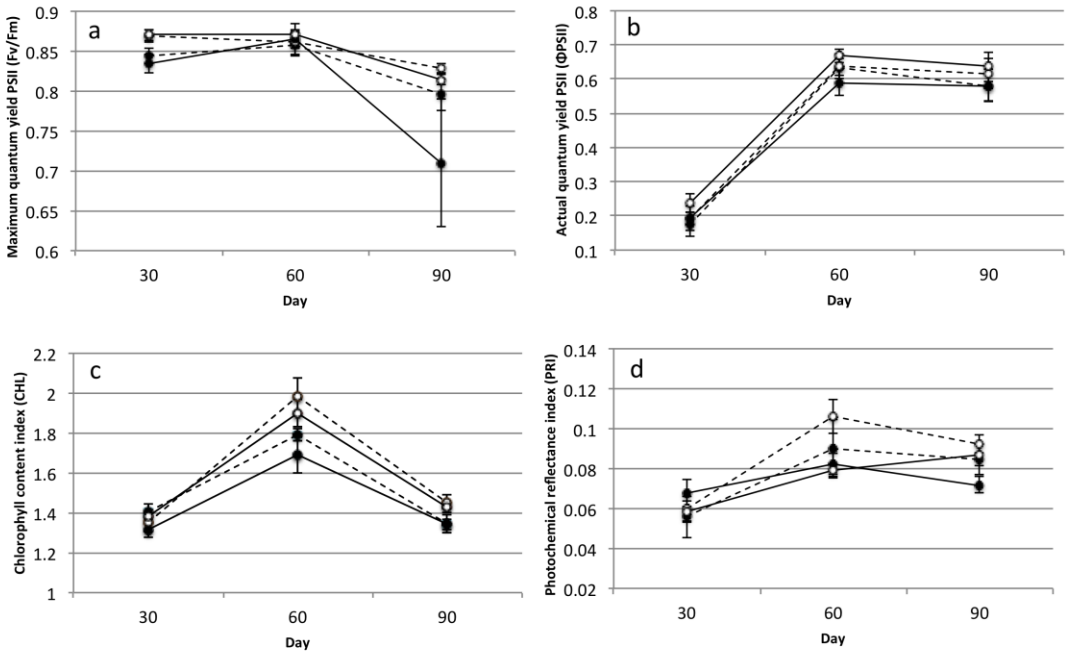

TABLE 1. Results of two-way analysis of variance (ANOVA) to examine the effects of stolon length and seawater submergence on total mass and biomass allocated to roots (root mass ratio, RMR). Values of $P < 0.05$ are in bold. See Fig. 2 for data.

Effects	Total mass			Root mass ratio (RMR)		
	df	*F*	*P*	df	*F*	*P*
Stolon	1	9.188	**0.004**	1	2.332	0.135
Submergence	1	66.044	**<0.001**	1	0.612	0.439
Stolon x submergence	1	1.902	0.176	1	0.006	0.939
Error	36			36		

TABLE 2. Results of two-way repeated-measure analysis of variance (ANOVAR) with stolon length and seawater submergence as between-subject effects for differences in maximum and actual quantum yield of PSII (Fv/Fm and ΦPSII, respectively), chlorophyll content index (CHL) and photochemical reflectance index (PRI). Values of $P < 0.05$ are in bold. See Fig. 3 for data.

	Fv/Fm			ΦPSII			CHL			PRI		
Between-subject effects	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Stolon	1	0.892	0.351	1	0.182	0.672	1	2.257	0.142	1	2.711	0.108
Submergence	1	4.738	**0.036**	1	2.016	0.164	1	10.544	**0.003**	1	1.245	0.272
Stolon x submergence	1	0.818	0.372	1	0.540	0.467	1	0.335	0.566	1	0.924	0.343
Error	36			36			36			36		
Within-subject effects												
Time	2	11.541	**<0.001**	2	26.845	**<0.001**	2	108.741	**<0.001**	2	17.334	**<0.001**
Stolon x time	2	1.571	0.215	2	0.432	0.651	2	0.016	0.542	2	2.462	0.092
Submergence x time	2	1.720	0.186	2	0.066	0.936	2	0.097	**0.031**	2	0.919	0.404
Stolon x submergence x time	2	0.555	0.576	2	0.267	0.766	2	0.014	0.588	2	0.884	0.417
Error	72			72			72			72		